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The Key Role of *Pseudomonas aeruginosa* PA-I Lectin on Experimental Gut-Derived Sepsis

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Objective

To examine the effect of *Pseudomonas aeruginosa* on intestinal barrier function and its lethal potential when introduced into the intestinal tract of mice.

Summary Background Data

The mere presence of *P. aeruginosa* in the intestinal tract of critically ill patients is associated with a threefold increase in death compared with matched cohorts without this pathogen. Whether this effect is a cause or a consequence of the critically ill state has not been previously addressed.

Methods

Transepithelial electrical resistance, a measure of tight junction permeability, was evaluated in Caco-2 intestinal epithelial cells cells apically inoculated with live *P. aeruginosa*, exotoxin A, or purified PA-I lectin, an adhesin of *P. aeruginosa*. Lethality studies to *P. aeruginosa* were carried out in mice undergoing 30% surgical hepatectomy by injecting the bacteria or its various components directly into the cecum.

Results

Only cells exposed to *P. aeruginosa* or its PA-I lectin developed alterations in barrier function. *P. aeruginosa* or the combination of PA-I and exotoxin A was lethal to mice when injected into the cecum after partial hepatectomy. Alterations in epithelial barrier function and death in mice were prevented when *Pseudomonas* was pretreated with *N*-acetyl D-galactosamine (GalNAc), a binder of PA-I.

Conclusions

P. aeruginosa may act as a pathogen in the gastrointestinal tract, resulting in altered epithelial barrier function and death in a susceptible host. The PA-I lectin of *P. aeruginosa* may play a key role in its pathogenicity to the intestinal epithelium by inducing a permeability defect to its cytotoxic exoproducts such as exotoxin A.

Although *Pseudomonas aeruginosa* is a feared hospital pathogen, it is considered to be part of the normal flora in healthy adults.[1] In fact, normal volunteers who swallow live cultures of *P. aeruginosa* remain healthy despite carrying the organism in their feces for up to 6 days.[2] In critically ill patients, however, the mere presence of *P. aeruginosa* in the proximal intestinal tract is associated with a 70% death rate, a threefold increase over age-matched critically ill patients who have negative cultures for this organism.[3] Although many have proposed that intestinal bacteria or their toxins can cause a state of systemic inflammation and sepsis, no pathogen or toxin has been identified that can independently cause this hypothetical state when introduced by means of the intestinal tract. When *P. aeruginosa*, however, is placed in the drinking water of immunosuppressed mice decontaminated of their indigenous flora with antibiotics, they die of gut-derived bacteremia.[4] This model does not explain the clinical observation that patients can die of sepsis presumed to be due to bacteria of gut origin while their cultures remain negative.

In the intestinal tract, the invasive and cytotoxic effects of bacteria are often judged by their ability to adhere to and alter the barrier function of cultured intestinal epithelial cells. We and others have shown that bacterial adherence alone can significantly alter the permeability of the intestinal epithelium to proinflammatory luminal macromolecules.[5,6] Recent evidence suggests that the PA-I lectin of

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P. aeruginosa may be an important component of its virulence phenotype by acting as an adhesin, facilitating its contact to vulnerable epithelial surfaces.[7] Therefore, the extent to which *P. aeruginosa* adheres to and alters the permeability of intestinal epithelia, the rate-limiting barrier of the intestinal mucosa, may have clinical relevance to its virulence.

Our laboratory has also established that the combination of a 30% surgical hepatectomy and short-term starvation alters the commensal *Escherichia coli* population in the cecum, resulting in an upregulation of their adherence phenotype.[8] We further established in this model that bacterial adherence to the cecal mucosa induced marked alterations in mucosal electrophysiology.[9] Therefore, the aims of this study were to characterize the effects of *P. aeruginosa* on the intestinal mucosal epithelium in human cultured epithelia, to determine its pathogenic effects in vivo when introduced into the mouse cecum, and to define the specific pathogenic role of PA-I lectin, a cytoplasmic adhesin of *P. aeruginosa*, in these systems.

METHODS

Cell Culture

All experiments were performed with Caco-2/C2bbe or "C2" cells (passage 51–68), which were a generous gift of Dr. Mark Mooseker (Yale University, New Haven, CT). C2 cells are a Caco-2 subclone that are highly differentiated intestinal epithelial cells.[10] C2 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY), 100,000 IU/L penicillin, 100 mg/L streptomycin, and 30 mg/mL human transferrin (Gibco BRL). C2 cells were grown at 37°C at 5% CO_2, but 24 hours before all experiments, cells were transferred to antibiotic-free conditions by using the same medium minus the penicillin/streptomycin. Cell viability was assessed by measuring lactate dehydrogenase release (Sigma, St. Louis, MO), as described by Tamai et al.[11]

Bacterial Strains/Components

ATCC strain 27853, a clinical isolate of *P. aeruginosa* from a blood culture, was used for all studies. This strain has been previously assessed for the presence of exotoxin A using both polymerase chain reaction and ADP-ribosylation assays.[12,13] The strain was assayed (erythrocyte agglutination assay) for the presence of PA-I and PA-II lectins using previously published methods.[14] The strain was found to have a PA-I concentration of 1:16 hemagglutination units. Overnight cultures of bacteria were grown in tryptic soy broth (Sigma) to the late logarithmic phase of growth, washed twice in phosphate-buffered saline (PBS), and resuspended to a final concentration of 5×10^7 colony-forming units (cfu/mL). Aliquots of bacteria were then placed in sterile cryovials and frozen with an equal volume of sterile 15% glycerol. For experiments, bacteria were thawed, centrifuged (9,000 rpm for 4 minutes), washed twice in PBS (pH 7.4) and resuspended in cell culture medium to a final concentration of 5×10^7 cfu/mL. Colony counts were routinely confirmed by diluting and plating samples on *Pseudomonas* isolation agar. Bacteria-free supernatant suspensions were obtained by centrifugation at 9,000 rpm for 4 minutes and passing the supernatant through a sterile 0.2-μm filter. Pseudomonal slime was obtained by differential centrifugation at 3,000 rpm for 10 minutes. A thick slime layer above the bacterial pellet was removed as the slime fraction after decantation of the supernatant. Formaldehyde killing of bacteria was carried out by suspending bacteria in a 3% solution for 1 hour, followed by vigorously washing of the bacteria in PBS, centrifugation, and resuspension in PBS. Purified PA-I and exotoxin A were obtained from Sigma.

Inoculation Onto Caco-2 Cells

Fifty microliters of live or formalin-fixed *Pseudomonas* in nonantibiotic cell culture medium was added to the apical side of confluent cell monolayers at a final concentration of 10^7 cfu/mL. Fifty microliters of bacterial components was similarly added to the apical side of the Caco-2 cells at the following concentrations: PA-I, 25 μg/mL; exotoxin A, 70 μg/mL; bacterial supernatant, 1:1 dilution; bacterial slime, 1:1 dilution. To determine the effect of the PA-I lectin of *P. aeruginosa* on transepithelial electrical resistance (TEER) in Caco-2 cells, both cells and bacteria were pretreated with the PA-I binding sugar *N*-acetyl-D-galactosamine (GalNAc) (w/v 3%).[15] Dextran (MW-4,000) (3% w/v), a nonspecific blocker of *Pseudomonas* adherence to epithelial cells, was also used to determine its inhibitory effect in this system.[16] For experiments assessing TEER, 50 μL of either GalNAc or dextran was added to the apical side of the transwells 24 hours before bacteria or bacterial component inoculation, and an equal volume of medium was withdrawn. Bacteria or PA-I was suspended in 50 μL of either GalNAc or dextran and added to the apical side of the cell monolayers 1 hour before TEER measurements.

Bacterial Adherence Assay

Dispersed Caco-2 cells were grown in T-75 flasks to a density of approximately 5×10^5 cells. *P. aeruginosa* was added to the Caco-2 cells at a final concentration of 10^7 cfu/mL in the presence or absence of 3% GalNAc, 3% dextran, or 5% mannose (w/v). The bacteria and Caco-2 cells were incubated at 4°C for 45 minutes by end-over-end rotation and then centrifuged at 900*g* for 10 minutes.[17] Bacterial adherence was expressed as the percentage of adherence of bacteria using the final colony count to the initial inoculum ratio.

Electrical Resistance Measurements and Mannitol Flux Studies

TEER was measured as previously described.[5] Transepithelial flux studies with the radioactive tracer [^{3}H] mannitol were used to determine whether the permeability defect was paracellular in selected experiments, according to previously published methods.[18] Briefly, cell monolayers in fibrillar collagen transwells were incubated under the experimental conditions in medium containing 5 mmol/L unlabeled mannitol in both the apical and basolateral reservoirs, and 1 μC/μL [^{3}H] mannitol (NEN, Boston, MA) was added to the apical surface after 4 hours of incubation with *P. aeruginosa* or PA-I in the absence or presence of GalNAc or dextran (3% w/v, 50 μL as in previous experiments). Unidirectional, mucosal-to-serosal, mannitol flux was measured during a 30-minute period.

Western Blot Analysis of Tight Junction Proteins

Levels of tight junctional proteins ZO-1, ZO-2, and occludin were analyzed by Western blotting using specific antibodies, according to previously published protocols.[19] Heat shock protein (HSP-72) was used as a positive control.

Mouse Studies

Mouse Strains/Housing

All experiments were approved by the Animal Care and Use Committee at the University of Chicago. Inbred mice of the Balb/c background weighing 20 to 25 g were used for all experiments. Mice were housed in individual wire-bottom cages to limit coprophagia during the entire experimental period.

Surgical Hepatectomy/Cecal Injection Method

For lethality experiments, mice were assigned to two surgical groups of either laparotomy only followed by direct cecal injection of *P. aeruginosa* or its components (control) or laparotomy + 30% surgical hepatectomy followed by direct cecal injection of *P. aeruginosa* or its components. Animals were allowed water ad libitum only for the remainder of the study period. Control animals were anesthetized (ketamine 100 mg/kg, xylazine 10 mg/kg, and atropine 0.04 mg/kg given intraperitoneally) and underwent a midline laparotomy incision and injection directly into the base of the cecum with 200 μL *P. aeruginosa* or its components at the following concentrations: live *P. aeruginosa*: 1×10^4 to 5×10^7 cfu/mL; PA-I: 1.33 mg/mL (undiluted 1:1 or diluted in PBS at 1:3 and 1:10); and exotoxin A: 0.33 mg/mL. After the injection and before removal of the needle, a fine silk suture was placed around the injection site and needle. As the needle was extracted, the suture material was tied, thereby sealing the puncture site. The puncture site was then swabbed with 70% isopropyl alcohol. This technique resulted in no leaks or evidence of local peritonitis after surgery. The hepatectomy was performed using an electrocautery device, excising the floppy left lobe of the liver, which resulted in minimal bleeding. Specimens were weighed and the ratio of excised liver to body weight was calculated to confirm uniformity of the resection.

To determine the systemic effect of live *P. aeruginosa*, PA-I, or exotoxin A on mouse death, 200 μL live *P. aeruginosa* (2×10^6 cfu/mL), PA-I (1.33 mg/mL), or exotoxin A (0.33 mg/mL) was injected intraperitoneally into 10 control mice and 10 mice undergoing 30% surgical hepatectomy. Mice were observed for 48 hours and death was recorded. All mice were killed at 48 hours, and the peritoneum was examined for evidence of local peritonitis. In selected animals, tissues (cecum, liver, blood) were harvested for bacterial culture and histologic examination.

Death Prevention Studies

Based on the dose-response experiments performed, animals undergoing hepatectomy and starvation were assigned to six groups of 10 animals each. Mice received 200 μL live *P. aeruginosa* at 2×10^6 cfu/mL or 200 μL of the combination of PA-I (1.33 mg/mL, diluted 1:2 in PBS) and exotoxin A (0.33 mg/mL) by the direct cecal injection method. Two additional groups underwent hepatectomy and had the identical doses of *P. aeruginosa* and PA-I and exotoxin A suspended in 13% GalNAc or 15% dextran for 20 minutes before cecal injection. To provide proliferating bacteria with a continued source of GalNAc and dextran, animals had 1 mL of each solution injected directly into the terminal ileum, which filled the small intestine proximally. To control for a possible dilutional effect alone, animals in the groups not receiving GalNAc or dextran had 0.9% NaCl similarly injected into the ileum. Animals were followed up for 48 hours and death was recorded. When the animals were killed, cultures were performed in selected cases.

Statistical Analysis

Data were loaded into the SigmaStat (Jandel Corp., San Rafael, CA) program and tested for significance using a one-way analysis of variance and Neuman-Keuls post hoc testing where appropriate. For nonparametric data involving the percentage (incidence) of death, the Fisher exact test was used. $P < .05$ was accepted for statistical significance.

RESULTS

P. aeruginosa and PA-I Alter the TEER of Caco-2 Monolayers in a Dose- and Time-Dependent Manner

Figure 1 demonstrates the effects of *P. aeruginosa* or its components on the TEER of Caco-2 monolayers measured at 2 and 4 hours after inoculation (triplicate cultures, n = 7 for each group). PA-I had an effect on TEER equal to the

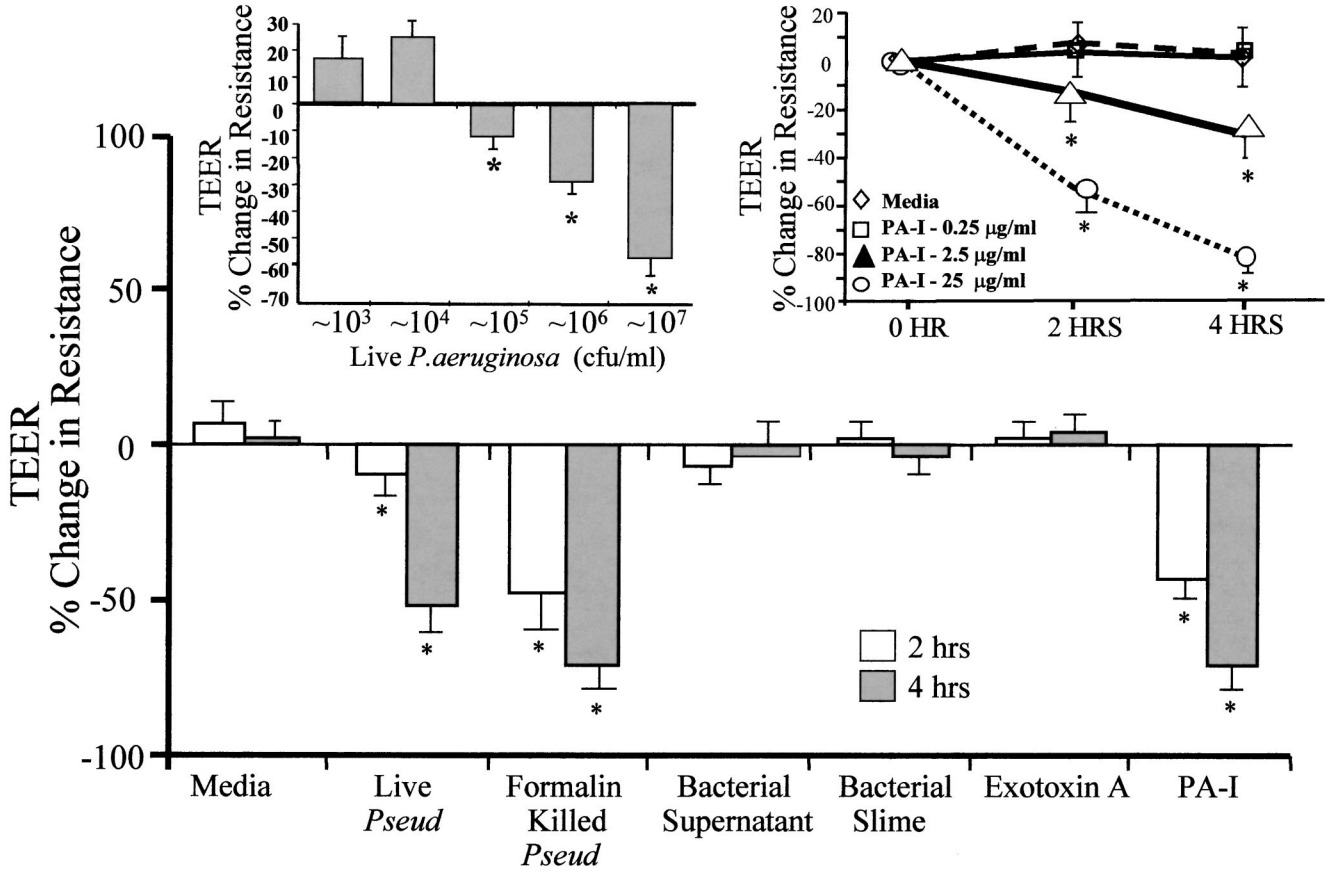

Figure 1. Percentage change in transepithelial resistance of Caco-2 epithelial monolayers exposed to *Pseudomonas aeruginosa* or its components at 2 and 4 hours after exposure. Data are means ± standard error of the mean of triplicate cultures (n = 7) at the time point specified. Dose-response data are mean ± standard error of the mean of duplicate cultures (n = 7) of resistance measured at 4 hours after exposure. Data demonstrate that both live and killed *P. aeruginosa* alter transepithelial resistance in a dose- and time-dependent manner. PA-I altered transepithelial resistance equal to that of whole bacteria; exotoxin A had no effect.

whole bacteria. No increase in lactate dehydrogenase release was observed in monolayers from the groups measured at 4 hours, suggesting that the monolayers were intact (data not shown).

P. aeruginosa Adherence to Caco-2 Cells and Its Effect on Barrier Function Are Inhibited by Dextran and GalNAc

Figure 2 demonstrates that 1% to 2% of the initial inoculating dose of *P. aeruginosa* adhered to Caco-2 monolayers (n = 7 per group; duplicate culture). This is in accordance with similar studies using enteric bacteria and human epithelial cells.[20] When bacteria were pretreated with mannose, an oligosaccharide that binds to the PA-II lectin of *P. aeruginosa*, a minimal decrease in adherence to Caco-2 cells was seen (data not shown). The effects of *P. aeruginosa*- and PA-I-induced responses in Caco-2 cells were studied in the absence and presence of GalNAc and dextran and are also shown in Figure 2 (n = 7 for each group; triplicate culture). Both GalNAc and dextran attenuated the decrease in TEER induced by live *P. aeruginosa* on Caco-2 monolayers ($P < .001$). GalNAc had a similar inhibitory effect on PA-I-induced decrease in TEER ($P < .001$). Dextran, however, had no effect on PA-I-induced decreases in TEER in this system.

Results of the mannitol flux analysis are shown in Figure 2 (n = 7 for each group of triplicate culture). Both *P. aeruginosa* and PA-I caused a significant increase in cumulative mannitol flux across Caco-2 monolayers ($P < .001$). Both GalNAc and dextran completely prevented the increased mannitol flux induced by either live *P. aeruginosa* or PA-I after 4 hours of incubation ($P < .01$).

P. aeruginosa Alters the Tight Junction Proteins ZO-1 and Occludin via Its PA-I Lectin

Results of the Western blot analysis of the tight junctional proteins ZO-1, ZO-2, and occludin are shown in Figure 3.

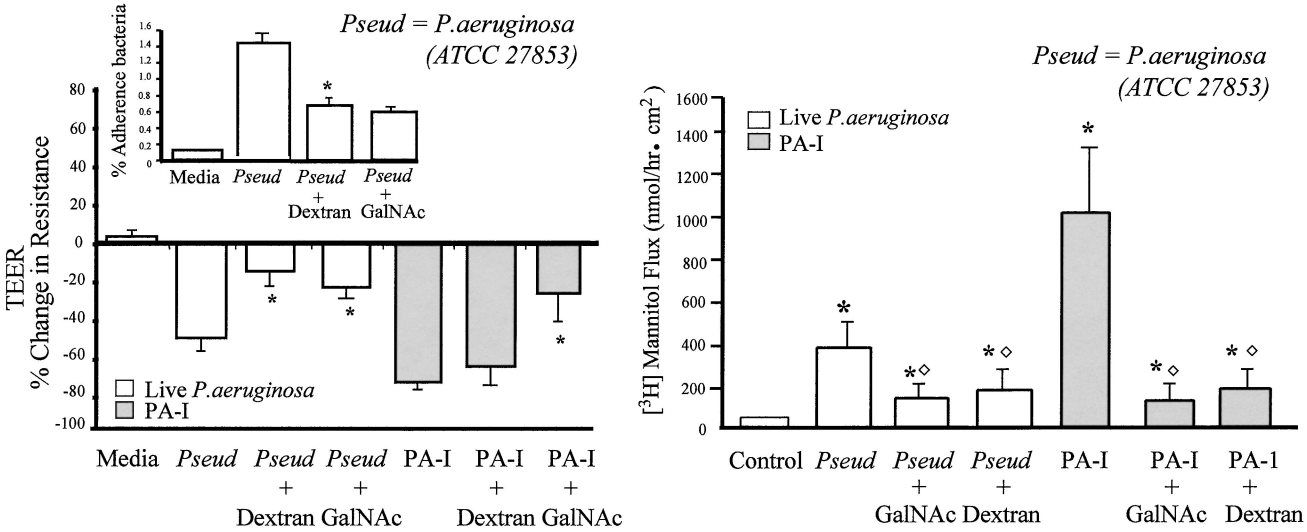

Figure 2. (Small graph) Percentage adherence of *Pseudomonas aeruginosa* (10^7 cfu/mL) to dispersed Caco-2 epithelial monolayers after 45 minutes of incubation at 4°C. Data are mean ± standard error of the mean of duplicate cultures (n = 7). (Large graph) Percentage change in transepithelial resistance of Caco-2 epithelial monolayers exposed to *P. aeruginosa* or its PA-I lectin. Experiments were performed in the absence and presence of GalNAc, a specific binder of PA-I, or dextran, a nonspecific inhibitor of *P. aeruginosa* adherence to epithelial cells and an epithelial membrane stabilizer. Data are mean ± standard error of the mean of triplicate cultures displayed at the 4-hour time point. As seen with other intestinal pathogens, approximately 1–2% of the initial inoculum of bacteria adhere to dispersed epithelial cells (see the small graph). Experiments with blocking compounds demonstrate that *P. aeruginosa* adherence to and alteration of the transepithelial resistance of Caco-2 cells are attenuated by the presence of either GalNAc or dextran. However, only GalNAc attenuated the decrease in resistance induced by PA-I. (Right Graph) [^{3}H]-mannitol flux (nmol/h · cm^2) across Caco-2 epithelial monolayers at 4 hours after incubation with *P. aeruginosa* or its PA-I lectin in the absence or presence of GalNAc or dextran. Data are mean ± standard error of the mean of triplicate cultures (n = 7). The apical exposure of either live *P. aeruginosa* or PA-I resulted in an increase in mannitol flux across the epithelial monolayer. Both GalNAc and dextran attenuated this effect.

Results of Mouse Studies

Live *Pseudomonas* or the combination of PA-I and exotoxin A injected into the cecum caused lethal sepsis in mice after hepatectomy. As shown in Figure 4, mice subjected to 30% hepatectomy and receiving a direct injection into the cecum of either live *P. aeruginosa* or the combination of PA-I and exotoxin A had a significant death rate.

Systemic administration of exotoxin A was lethal, but PA-I had no effect. Table 1 demonstrates that *P. aeruginosa* adheres to the cecum of mice undergoing hepatectomy to a greater extent ($P < .01$) than to the cecum of control mice. Dissemination of *P. aeruginosa* to the liver and blood was not quantitatively different between control and hepatectomy mice. The administration of exotoxin A to mice by the intraperitoneal route resulted in 100% death at 48 hours, whereas PA-I had no effect, independent of hepatectomy. These findings suggest the possibility that exotoxin A absorption from the cecum may be necessary to induce death in this model, whereas the effect of PA-I absorption is negligible. In marked contradistinction to intracecal injection, intraperitoneal injection of *P. aeruginosa* resulted in no deaths in control or hepatectomy mice. Both groups of mice developed bacteremia with *P. aeruginosa* after intraperitoneal injection (data not shown).

Death prevention experiments are summarized in Figure 5. GalNAc completely prevented death in mice after hepatectomy and cecal injection of live *P. aeruginosa*. Death was also prevented with GalNAc pretreatment in mice who received the combination of PA-I and exotoxin A. Dextran only partially attenuated death in this model, and it had no effect on death prevention in mice injected with both PA-I and exotoxin A.

DISCUSSION

P. aeruginosa is a feared hospital pathogen because of its high antibiotic resistance capacity and its lethal virulence characteristics. This pathogen tends to predominate on the mucosal surfaces of the most immunologically compromised hospital patients and is associated with a high death rate.[21,22] Among patients undergoing chemotherapy who subsequently become bacteremic with *P. aeruginosa*, 81% were fecal carriers of the same strain,[23] suggesting that in some instances, the intestinal tract may be a reservoir for this pathogen. Little information is available, however, on the effects of this organism on the intestinal mucosa, because it is not considered to be an intestinal pathogen.

One particularly well-studied effect of pathogenic bacteria on the intestinal mucosa is their ability to alter the tight

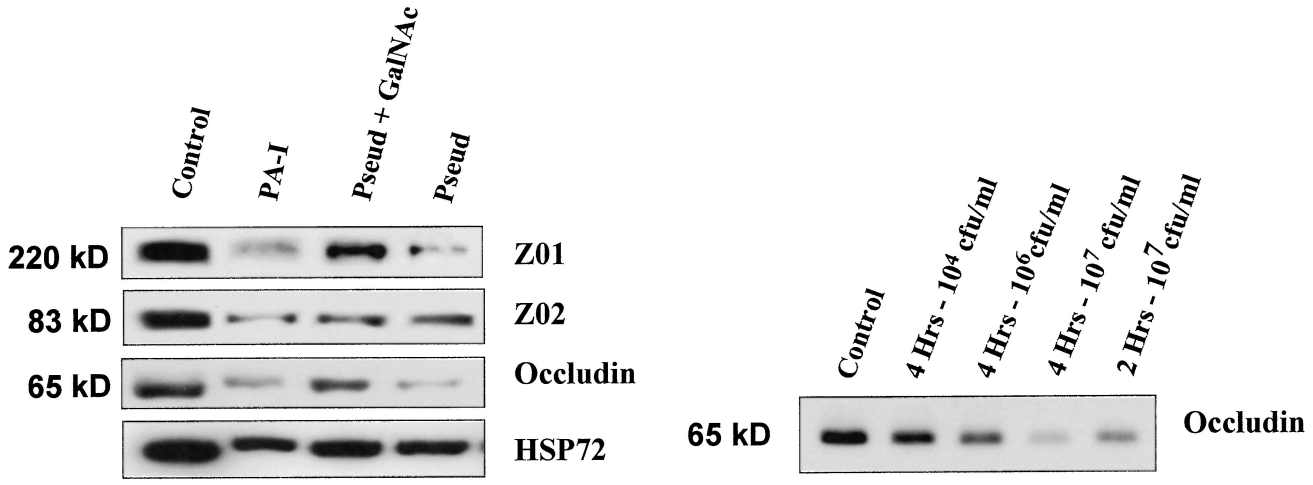

Figure 3. Western blot analysis of the effect of live *Pseudomonas aeruginosa* or PA-I on the tight junctional proteins ZO-1, ZO-2, and occludin. Live *P. aeruginosa* was pretreated with 2% GalNAc. Dose and time responses of occludin after exposure to live *P. aeruginosa* was assessed. Heat shock protein (HSP-72) was also measured as a positive control. Results suggest that *P. aeruginosa* alters both ZO1 and occludin in Caco-2 cells. That this effect is mediated by its PA-I lectin is suggested by experiments using PA-I alone and by blocking experiments with GalNAc, where attenuation of the effect is seen. As with functional experiments, this effect appears to be both dose- and time-dependent

junction permeability or barrier function of the lining mucosal epithelial cells. Bacteria-induced alterations in epithelial permeability have been demonstrated for enteroinvasive strains of *E. coli* and *Salmonella typhimurium*.[24] Recent data from these in vitro studies suggest that bacteria-induced alterations in tight junction permeability occur by means of contact-dependent signal transduction events.[25] However, the specific virulence determinants on bacteria responsible for the initial contact with epithelia are unknown. Many soluble bacterial toxins such as lipopolysaccharide (endotoxin) from various *Enterobactericiae* species do not alter intestinal epithelial permeability when applied locally, nor do they have other pathologic effects, such as the release of interleukin-8 or tumor necrosis factor α.[26,27] Because gram-negative bacteria constantly shed their outer membrane fragments during growth, endotoxins (lipopolysaccharide) are in abundant supply in the intestinal lumen.[28] It has been proposed, but not proved, that during critical illness, intestinally derived toxins from colonizing but otherwise apathogenic bacteria (to the intestinal mucosa) can cause systemic sepsis through histologicaly intact intestine and in the absence of an intestinal inflammatory response.[29]

Results of the mice studies in this report suggest that the PA-I lectin of *P. aeruginosa* may be a key component necessary for lethal infection originating from the intestines of a susceptible host. A susceptible host is defined in the present study as a 30% hepatectomy—a mild, well-tolerated surgical procedure that results in high survival rates when no infectious challenge is administered. Data from the present study would suggest that live cells of *P. aeruginosa*, or the combination of PA-I and exotoxin A, cause an increased death rate in mice undergoing hepatectomy as a result of the immunocompromise of the host, because equal inocula of bacteria or bacterial components resulted in low death rates in control mice. The observation that systemic injection of *P. aeruginosa* resulted in no deaths in either group of mice, coupled with the observation of equal quantitative dissemination of bacteria to the liver and blood of both groups, despite different death rates, suggests an alternative explanation. The observation that intestinal introduction of *P. aeruginosa* carries a higher death rate than systemic administration was previously reported by Schook et al.[30] Given that bacteria can express or repress virulence determinants depending on their environment and tissue site, it is possible that the intestinal tract of mice undergoing hepatectomy and starvation upregulates the virulence potential of these bacteria. Subsequent work from our laboratory with this mouse model has demonstrated that hepatectomy and starvation results in significant alterations in the physical microenvironment of the cecum, with alterations in luminal pH and redox potential and elevated luminal norepinephrine concentration.[31] All three of these parameters have been demonstrated independently to induce virulence expression in bacteria in culture.[32] We have further demonstrated in this model that stock strains of *P. aeruginosa* express more PA-I and virulence to cultured intestinal epithelial cells when grown in a medium rich in norepinephrine (Alverdy JC, unpublished observations). Therefore, firm experimental evidence is accumulating that suggests that the enhanced death rate after hepatectomy and cecal injection of *P. aeruginosa* is a result of bacterial virulence phenotype transformation in response to luminal norepinephrine. This hypothesis fits nicely with current paradigms of bacterial virulence transformation in response to environmental cues, as proposed by Mekalanos.[33]

It seems likely that the observed effects of *P. aeruginosa*

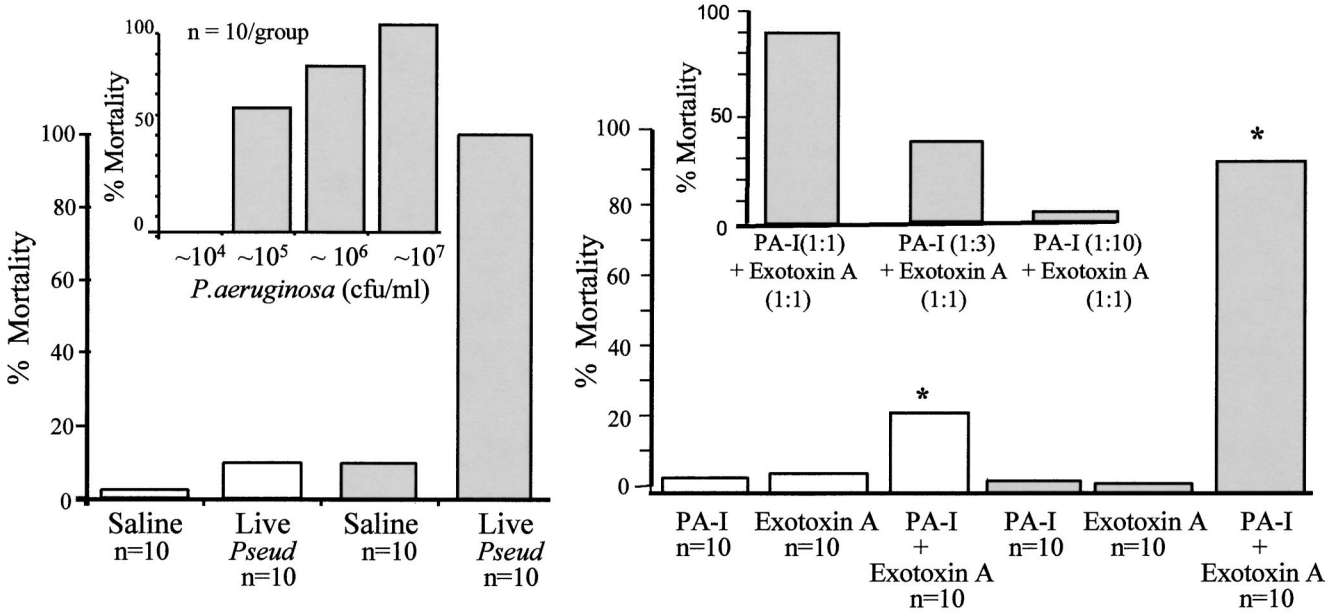

Figure 4. Death rate of mice subjected to sham laparotomy or 30% surgical hepatectomy and receiving direct injection into the cecum of live cultures of *Pseudomonas aeruginosa*, saline, or virulence components. Death is defined as the percentage of mice dead at 48 hours after bacterial exposure. Results demonstrate that cecal injection of greater than 10^5 cfu/mL of *P. aeruginosa* results in a significant (90%) death rate after a 30% surgical hepatectomy. Only the combination of PA-I and exotoxin A induced death in mice; this was greater for mice after hepatectomy (*$P < .001$). Dose-response experiments and the lack of death with exotoxin A alone suggest that PA-I is a key element in this response.

on mouse death were in part mediated by both PA-I and exotoxin A, because the latter combination effectively resulted in similar death rates compared with whole bacterial cells. Further, the finding that only GalNAc completely prevented death in this model suggests a major role for PA-I in gut-induced sepsis from *P. aeruginosa*. The lack of effectiveness of dextran in this model may be related to the amount of freely secreted PA-I and exotoxin A that is released in the cecum during infection. PA-I lectin is reported to be present in *P. aeruginosa* culture medium at a concentration greater than 20 μg/mL for a bacterial inoculum of 10^9 bacteria per milliliter. PA-I lectin has also been detected at higher concentrations in infected sputum of cystic fibrosis patients.[34] The dose of PA-I injected intraluminally that would approximate the concentration presented to its receptors on epithelial cells from adherent *P. aeruginosa* is difficult to estimate, and therefore the physiologic relevance of the doses of PA-I chosen in the present study will need to be clarified.

Based on the results of the Caco-2 experiments, we would speculate that PA-I induces a permeability defect of sufficient magnitude to permit the permeation of exotoxin A systemically in mice. The finding that PA-I injected systemically (intraperitoneally) into mice does not induce death or any appearance of illness, whereas exotoxin A does, suggests that the mechanism of lethality in this model is by a PA-I-mediated permeability defect to exotoxin A. The precise mechanism by which PA-I and exotoxin A injection into the cecum of mice after hepatectomy causes death remains to be clarified.

Data from the Caco-2 experiments suggest that live or formalin-killed *P. aeruginosa* induces a defect in epithelial

Table 1. LOCAL VS. SYSTEMIC EFFECT OF *P. AERUGINOSA* OR ITS COMPONENTS

	Quantitative Bacterial Culture			Incidence of Death With Systemic Injection		
Direct Cecal Injection 200 μL P. aeruginosa (~2 × 10^6 cfu/mL)	Cecum (cfu/g–$\log_{10}$)	Liver (cfu/g–$\log_{10}$)	Blood (cfu/mL–$\log_{10}$)	PA-I, Intraperitoneal (200 μL–1.33 mg/mL)	Exotoxin A, Intraperitoneal (200 μL–0.33 mg/mL)	200 μL (~2 × 10^6 cfu/mL) live *Pseudomonas*, Intraperitoneal
Control (n = 5)	2.81 ± 0.13	2.71 ± 1.6	2.14 ± 0.6	0/5	5/5	0/5
30% hepatectomy (n = 5)	4.14 ± 0.23*	2.98 ± 0.21	1.96 ± 0.5	0/5	5/5	0/5

*$P < .001$ vs. control.

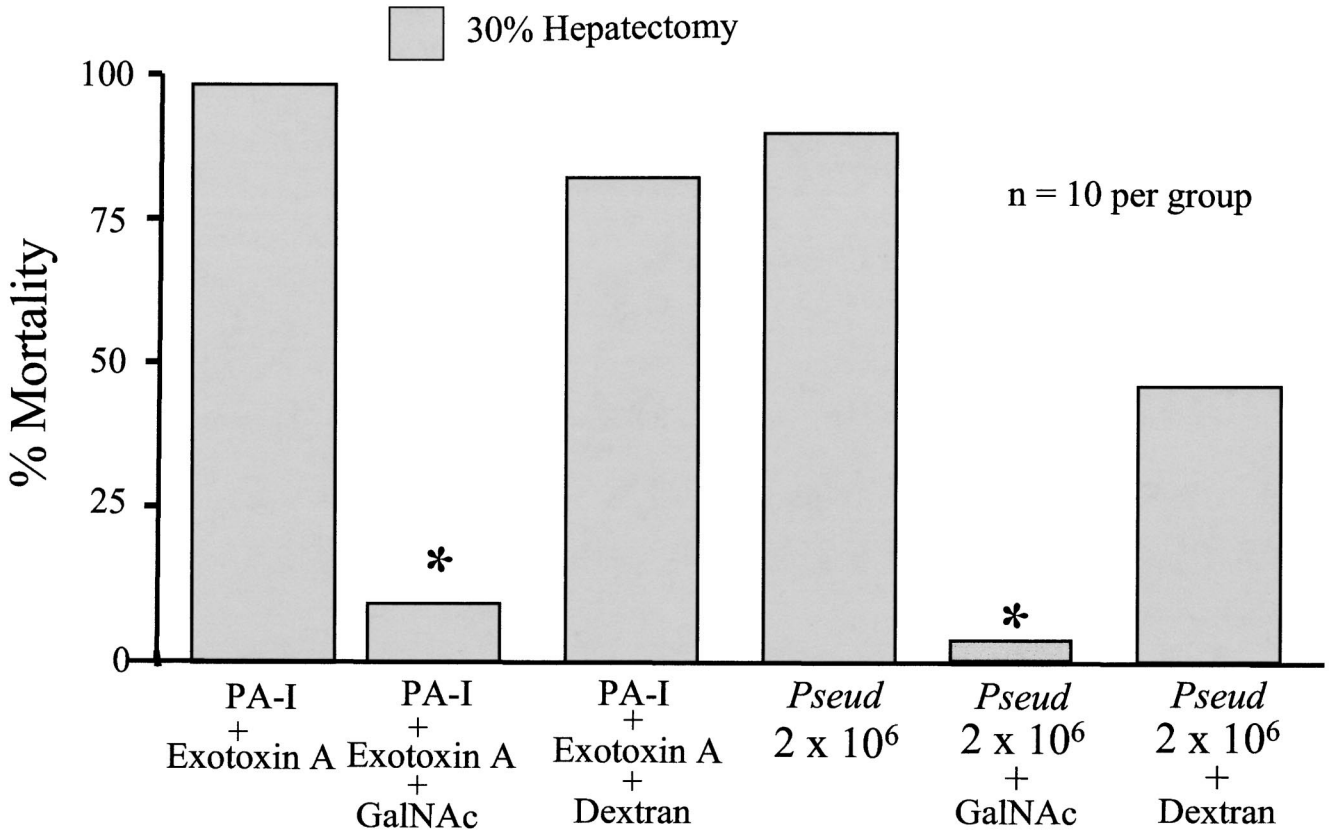

Figure 5. Death rate of mice after 30% hepatectomy and injection of either live *P. aeruginosa* or the combination of PA-I and exotoxin A in the presence and absence of 13% GalNAc or 15% dextran. Results demonstrate that although both GalNAc and dextran appeared to protect against death in this model, only GalNAc resulted in a statistically significant and complete prevention of death in mice injected intracecally with either live *P. aeruginosa* or the combination of PA-I and exotoxin A (*$P < .001$).

permeability at the level of the intercellular tight junctions, based on the transepithelial flux of mannitol, a paracellular permeability probe. We have previously demonstrated that live *P. aeruginosa* alters the tight junction regulatory protein ZO-1 in T-84 human colon epithelia.[35] Western blot analysis of ZO-1 and occludin in epithelial cells exposed to live *P. aeruginosa* with and without GalNAc suggests that this effect in part involves a disruption in tight junctional proteins. Data from the present study also demonstrate a rapid (<4 hour) decrease in TEER when Caco-2 cells were exposed to either whole cells of *P. aeruginosa* or PA-I. Previous time-response studies examining bacterial-induced alterations in barrier function report a time requirement of at least 8 to 10 hours of incubation to decrease TEER by 30%, reaching a maximal decrease in TEER at 24 hours of 50%.[36] Defined in terms of rapidity and severity of the permeability defect, *P. aeruginosa* is among the most pathogenic organisms to cultured intestinal epithelia reported to date.

Dextran and GalNAc were chosen for inhibition experiments because dextran has been previously shown to block *P. aeruginosa* adherence to epithelial cells by an unknown mechanism, and GalNAc is known to bind specifically to PA-I.[15,16] Both dextran and GalNAc inhibited the adherence of *P. aeruginosa* to Caco-2 cells to a similar degree of less than 10^5 cfu/mL. This degree of adherence was below the threshold concentration necessary for changes in permeability to occur based on dose-response experiments. This is consistent with the reported function of PA-I as a mechanism by which *P. aeruginosa* adheres to intestinal epithelial cells (Alverdy JC, unpublished observations). That PA-I is responsible for *P. aeruginosa*-induced alterations in TEER and mannitol flux is inferred from the inhibition studies with GalNAc. Because PA-I alone can induce changes in TEER and mannitol flux equal to whole bacteria, this, coupled with GalNAc inhibition studies, suggests a major role for PA-I in this response. Data from these experiments also demonstrated that although both GalNAc and dextran attenuate *P. aeruginosa*-induced decreases in TEER, only GalNAc inhibited PA-I-induced decreased TEER; dextran had no effect. Increased mannitol flux in Caco-2 cells exposed to either live *P. aeruginosa* or PA-I, in contrast, was completely prevented with GalNAc or dextran treatment. TEER to passive ion flow is an extremely sensitive measure of barrier function, whereas mannitol permeation across Caco-2 cells must conform to the Stokes radius and possibly the charge of the paracellular pathway. It is not known how dextran inhibits the adherence of *P. aeruginosa* to epithelial cells. Labeling studies have demonstrated that dextran nei-

ther adheres to the epithelial cells nor the bacteria.[16] Dextran, however, is known to change the surface hydrophobicity and charge of epithelial cells and may result in steric hindrance or electrostatic shielding of the cell glycocalyx.[37] Dextran has been shown to block mannitol permeability completely in cultured epithelial cells exposed to elastase, and it prevented intestinal barrier dysfunction after experimental hepatectomy in rats.[38,39] The fact that tight junctions are known to discriminate both by charge and size could explain the finding that dextran prevented PA-I-induced alterations in mannitol flux without affecting TEER.

P. aeruginosa may fulfill the requirements for inducing a state of gut-derived sepsis with or without the translocation of the whole bacterial cells. Because of its known properties of immunoevasion and biofilm production, this organism may be potentially harmful if specific phenotypes are expressed in the mammalian intestine in a susceptible host. The PA-I lectin of *P. aeruginosa* is of particular interest because it can switch to the "on/off" variation or to the "in/out" position on the outer surface of the cell, depending on the environmental cues it receives.[40] These adaptive features of the lectins of *P. aeruginosa* help regulate bacterial adaptability, whereby access to host cells for nutritional purposes, self-protection, or attack is possible. In the context of the present set of mouse experiments, it is possible that the cecum is used as an immunologically privileged site for *P. aeruginosa*, whereby it induces a permeability defect (by PA-I) to its cytotoxins while remaining inaccessible to the host defense tactics. Once bloodborne, however, PA-I expression may be downregulated, because this phenotype represents a liability to the organism: it is also a ligand for neutrophil attachment. Because the expression of PA-I is coregulated with exotoxin A and is located together with other cytotoxic exoproducts on the *P. aeruginosa* virulence island gene, bloodborne organisms have downregulated virulence and by themselves do not induce death.[41–43]

In summary, we established that the PA-I lectin of *P. aeruginosa* is important for adherence to intestinal epithelial cells and can significantly perturb their barrier function. We speculate that in the intestinal tract, the mere presence of exotoxin A-producing strains of *P. aeruginosa* expressing the PA-I lectin has the potential to be of pathogenic importance in the critically ill.

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